

**CLAIMS**

1. An antibody Fab or Fab' fragment to which at least one effector molecule is attached characterized in that the heavy chain in the fragment is not covalently bonded to the light chain and both the interchain cysteine of C<sub>L</sub> and the interchain cysteine of C<sub>H1</sub> have been replaced with another amino acid.
2. The antibody fragment of claim 1 wherein the interchain cysteine of C<sub>L</sub> and the interchain cysteine of C<sub>H1</sub> have been replaced with a non-thiol containing amino acid.
3. The antibody fragment of claim 2 wherein the interchain cysteine of C<sub>L</sub> has been replaced with serine.
4. The antibody fragment of claim 2 wherein the interchain cysteine of C<sub>H1</sub> has been replaced with serine.
5. The antibody fragment of claim 2 wherein both the interchain cysteine of C<sub>H1</sub> and the interchain cysteine of C<sub>L</sub> have been replaced with serine.
6. The antibody fragment of claims 1-5 wherein the interchain cysteine of C<sub>L</sub> is at position 214 of the light chain and the interchain cysteine of C<sub>H1</sub> is at position 233 of the heavy chain.
7. The antibody fragment of claims 1-6 where at least one effector molecule is attached to the heavy or light chain constant region.
8. The antibody fragment of claims 1-7, wherein an effector molecule is attached to a cysteine in the light chain constant region and a cysteine in the heavy chain constant region.
9. The antibody fragment of claim 8, wherein the cysteine residues in the heavy and light chain constant regions which are attached to effector molecules would otherwise be linked to each other via a disulphide bond if the effector molecules were not attached.
10. An antibody Fab' fragment according to claims 1-9 that contains a modified hinge region.
11. An antibody Fab' fragment according to claim 10 in which the modified hinge contains 1 cysteine residue.
12. An antibody Fab' fragment according to claim 11 in which the hinge comprises the sequence in SEQ ID NO:1 or SEQ ID NO:2.

13. The antibody fragment of claim 10 in which the modified hinge contains 2 cysteine residues.
14. An antibody Fab' fragment according to claim 13 in which the hinge comprises the sequence in SEQ ID NO:3 or SEQ ID NO:4.
15. An antibody Fab' fragment according to claims 1-14 where at least one effector molecule is attached to the hinge region.
16. An antibody Fab' fragment according to claim 15 in which two effector molecules are attached to the hinge region.
17. An antibody Fab' fragment according to claims 1-16 in which all the effector molecules attached to the fragment are attached to the hinge region.
18. An antibody Fab' fragment according to claims 1-17 in which each effector molecule attached to the fragment is attached to a cysteine in the hinge region.
19. A method of producing an antibody Fab or Fab' fragment according to claims 1-18 comprising:
  - a. treating an antibody Fab or Fab' fragment in which both the interchain cysteine of C<sub>L</sub> and the interchain cysteine of C<sub>H1</sub> have been replaced with another amino acid with a reducing agent capable of generating at least one free thiol group in the fragment
  - b. reacting the treated fragment with an effector molecule
20. The method according to claim 19 in which the reductant is a non-thiol based reductant.
21. The method according to claim 20 in which the reductant is a trialkylphosphine.
22. The method according to claim 21 in which the trialkylphosphine reductant is tris(2-carboxyethyl)phosphine (TCEP).
23. The method according to claim 22 in which the trialkylphosphine reductant is tris(3-hydroxypropyl)phosphine (THP).
24. The method according to claim 23 in which either or both of steps (a) and (b) are performed in the presence of a chelating agent.
25. The method according to claim 24 in which the chelating agent is EDTA.
26. The method according to claim 25 in which both steps (a) and (b) are performed in the presence of EDTA.
27. A mixture containing two or more Fab or Fab' fragments, characterized in that the mixture is enriched for Fab or Fab' fragments in which the light chain in said fragments is not covalently bonded to the heavy chain, both the interchain cysteines

of  $C_L$  and  $C_{H1}$  have been replaced by another amino acid and at least one effector molecule is attached to the fragment.

28. A mixture according to claim 27 in which greater than 50% of the mixture comprises a Fab or Fab' fragment in which the light chain in said fragment is not covalently bonded to the heavy chain, both the interchain cysteines of  $C_L$  and  $C_{H1}$  have been replaced by another amino acid and at least one effector molecule is attached to the fragment.
29. The antibody fragment of claims 1-28 wherein the effector molecule is PEG.
30. A pharmaceutical composition comprising an antibody fragment according to any of the preceding claims, together with one or more pharmaceutically acceptable excipients, diluents or carriers.